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(54) **Method and apparatus for controlling feed of incubating substrate.**

(57) In incubating an enzyme under aerobic condition, a substrate feeding speed of a substrate feed quantity to an incubation tank per unit quantity of a fungus body (specific feeding speed; hereinafter referred to as an "value") is controlled. When no by-product is detected, the α value is increased at a predetermined rate.

When the by-products are detected, the α value is decreased at a rate of greater than the above predetermined rate.

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Title of the Invention

Method and apparatus for controlling feed of incubation substrate

Background of the Invention

This invention relates to a method of controlling the incubation of bacteria under aerobic condition. More particularly, the present invention relates to a method and apparatus for controlling the substrate feeding quantity during incubation.

In producing enzymic fungus bodies such as yeast for foodstuffs and livestock feed and bread yeast, feeding culture has been employed in which small amounts of a substrate is sequentially fed to the medium. To efficiently carry out the incubation, it is essential that the substrate fed to the medium be completely consumed by the yeast and formation of by-products such as ethanol be less.

No method has yet been established which radpidly measures the concentration and activity of the fungus body during incubation. For this reason, the substrate feed system which feeds the substrate to the incubation tank in accordance with a predetermined program has been used. This system can not feed the substrate in accordance with the activity of the fungus body inside the tank and hence, has low producibility.

Summary of the Invention

The present invention is directed to provide a method and apparatus for improving the production efficiency of the fungus body of the enzyme by suitably controlling the substrate feeding quantity during incubation.

To accomplish the object, the substrate feeding method of the present invention is characterized in that formation of by-products during incubation is detected using a substrate feeding speed of a substrate feed quantity to an incubation tank per unit quantity of a fungus body (specific feeding speed; hereinafter referred to as an "α value") as a parameter, the α value is increased at a predetermined rate when no by-product is detected, and when the by-products are detected, the α value is decreased at a rate greater than the predetermined rate described above.

Brief Description of the Drawings

Figures 1 through 6 are diagrams showing the relation between the varying α values and the ethanol formation;

Figures 7 and 8 are schematic views, each showing an example of the incubation apparatus of the present invention;

Figure 9 is a diagram showing the example of incubation in accordance with the present invention; and

Figure 10 is a diagram showing an example of estimation of the fungus body in accordance with the present invention.

Detailed Description of the Preferred Embodiments

The substrate feeding method in accordance with the present invention will be described in detail. First, the α value can be expressed by the following formula:

$$\alpha = \frac{F\ So}{V\ X} \qquad \cdots\cdots\cdots\cdots \quad (1)$$

where F is a feeding speed (ℓ/hr), So is a substrate concentration (g/ℓ),

V is a quantity of the culture solution and X is a concentration of the fungus body.

From the formula (1), the feeding speed F can be set in accordance with the following formula by determining α , V and X:

$$F = \alpha \cdot \frac{V X}{So} \quad \text{..... (2)}$$

The α value, or the substrate feeding speed per unit quantity of the fungus body, changes with the activity of the enzyme employed. In the case of the bread yeast, the yeast can be multiplied by the substrate ingested by raising the α value and increasing the substrate feeding speed if the activity is high. If the activity is low, on the other hand, the ingested substrate changes into ehtanol and CO_2 by the glucose effect, thus lowering the producibility.

The activity of the enzyme means the capacity of the enzyme that completely consumes the substrate fed and produces the fungus body without forming by-products such as ethanol. Hence, no method is available to directly measure the activity and after all, it is inevitable to employ a method which feeds the substrate so as not to form the by-products. Though a method of estimating the enzymic activity is available by use of the oxygen consumption speed of the enzyme as a parameter, the oxygen consumption speed is closely related with, and changes, with the substrate feeding speed. For this reason, this method alone is not sufficient.

Accordingly, the present invention proposes a method which sets an initial value of α at the start of incubation, then feeds the substrate at a feeding rate corresponding to the set α value and raises the α value if no by-products are formed. In this case, the raising rate of the α value

becomes a serious problem but as a result of intensive investigation, the inventors of the present invention have found a method which raises the value at the rate of up to 20 % and preferably 10 %, as the value matching with the change in the physiological activity of the enzyme.

The α value is raised at a rate of up to 20 % because the feed of substrate becomes excessive if the rate is more than 20 %. If the raising rate is too small, on the other hand, the substrate feed becomes insufficient and producibility of the fungus body can not be improved. Hence, the preferred raising rate is set to 10 %.

If the by-products are formed, the substrate feeding speed must be lowered, otherwise the by-products are formed continuously and the producibility of the fungus body drops. However, the formation of the by-products can not be stopped if the α value is reduced at the same rate as the raising rate. This is because the mechanism of the multiplication of the fungus body by decomposition of the substrate is different from the mechanism of the formation of the by-products. It is assumed that once the enzymic activity of the mechanism of the formation of the by-products becomes high, the flow of metabolic products is directed to the formation of the by-products. Accordingly, the reducing rate of substrate feed becomes important. Investigation has revealed that the formation of the by-products is stopped and the by-products formed in the culture solution can be used again by the enzyme if the reducing rate of the α value is at least 30 % and preferably, 50 %.

Figures 1, 2 and 3 illustrate examples of experiments of various substrate feeding systems using the bread yeast. In these examples, feed-back control by the respiratory quotient (RQ) was started from 1 or 2 hours after the start of incubation and the α value was changed every 15 minutes

so as to control the substrate feeding speed.

Figure 1 illustrates the system in which the α value was raised at a rate of 10 % and was reduced at a rate of 10 %. When the control range of RQ was from 0.8 to 1.0 mol/mol, RQ exceeded 1.0 mol/mol when ethanol was formed in an amount of about 1 g/ℓ. From this point the α value was reduced at a rate of 10 % but the formation of ethanol was not stopped and about 15.4 g/ℓ of ethanol was eventually formed. It is assumed that once ethanol is thus formed, the fermentation physiology of the enzyme reaches a state which is suitable for the ethanol formation and the physiological condition can not be rapidly changed to a state suitable for the multiplication of the fungus body if the reducing rate of the α value is small.

Figure 2 illustrates the system in which the α value was raised at a rate of 20 % and was reduced at a rate of 20 %. When the control range of RQ was from 0.8 to 1.0 mol/mol, RQ exceeded 1.0 mol/mol when 8.4 g/ℓ of ehtanol was formed. It is assumed that ethanol was formed in a greater amount than in the case of Figure 1 because the raising rate of the α value was greater. Though the α value was thereafter reduced at a rate of 20 %, the ethanol formation was not stopped and 14.7 g/ℓ of ethanol was eventually formed.

Figure 3 illustrates the system in which the α value was raised at a rate of 50 % and was reduced at a rate of 50 %. When the control range of RQ was from 0.9 to 1.0 mol/mol, RQ exceeded 1.0 mol/mol at the point when about 1.5 g/ℓ of ethanol was formed. Though the α value was repeatedly raised and reduced, the ethanol formation was not stopped and 10.5 g/ℓ of ethanol was eventually formed. In addition, RQ caused hunching.

It has been found from the experiments described above that the raising

rate of the α value must be up to 20 % because if it is 50 %, RQ causes hunching. However, the raising rate of 10 % is the most suitable rate because the formation quantity of ethanol is great if the rate is 20 %. On the other hand, the ethanol formation can not be inhibited if the reducing rate of the α value is below 20 %.

In Figures 1 and 2, ehtanol was seen formed even if the α value did not change. Figure 4 shows the result of an experiment carried out to confirm this phenomenon. When the substrate was fed at a substantially constant value, i.e., 0.3 g/g·h in RUN 1, 0.39 g/g·h in RUN 2 and 0.42 g/g·h in RUN 3, the ethanol formation was seen occurring at 5 hour, 7 hour and 11 hour in the order of higher α values. This means that the physiological activity of the enzyme changes even if substrate feeding is effected with a constant α value. It is therefore assumed that the reason why the ethanol formation could not be stopped by merely returning the α value to the original α value before ethanol was formed is that the change has occurred in the physilogical activity of the enzyme, that is, the ethanol formation has a a time lag.

Next, Figure 5 illustrates the result of investigation of the reducing rate of the α value. Incubation was started under the state in which 12.7 g/ℓ of ethanol was present at the initial stage of incubation. In this case, too, ethanol could not be reduced, though the α value was reduced at a rate of 20 % at the point when RQ exceeded 1.0 mol/mol. When the α value was reduced at a rate of 50 % at 4 hour from the start of incubation, ethanol was reduced drastically, and could be reduced to 2 g/ℓ, though the α value was thereafter raised at a rate of 20 %. It can be seen from the above that if the reducing rate of the α value is 20 %, the rise of the ethanol concentra-

tion in the culture solution can be prevented but ehtanol can not be reduced. However, if the reducing rate is 50 %, the physiological activity of the enzyme can be changed so that the ethanol formation can be checked and ethanol consumption takes place. It has thus been found that the reducing rate of the α value is at least 30 % and preferably, 50 %.

Figure 6 illustrates data when the α value was raised at a rate of 10 % and was reduced at a rate of 30 %. It can be understood that though the effect can be observed at the reducing rate of 30 %, it is considerably inferior to the reducing rate of 50 %.

As described in the foregoing, the inventors of the present invention have found that the effective system is one that raises the α value at a rate of up to 20 % and preferably, 10 % and reduces the α value at a rate of at least 30 % and preferably 50 %, if the by-products are formed, in order to change the physiological state of the enzyme from the formation of the by-products to the multiplication of the fungus body.

To practise the present invention, the quantity of the fungus body inside the incubation tank must be measured but no effective method has yet been found to directly measure the quantity of the fungus body. Accordingly, the inventors of the present invention have found that the quantity of the fungus body can be reasonably estimated from (1) oxygen incomings and outgoings, (2) carbon incomings and outgoings and (3) multiplication model.

Estimation of the quantity of the fungus body by the oxygen incomings and outgoings can be expressed by the following formula:

$$\triangle X = (a_2 \cdot \triangle s - \triangle O_2 - a_3 \cdot \triangle P)a_1 \quad \ldots\ldots\ldots\ldots (3)$$

where $\triangle X$ is a multiplied quantity of the fungus body (g), $\triangle s$ is a

substrate feeding quantity (g), O_2 is an oxygen consumption quantity (mol), P is a by-product quantity (g), a_1 is an oxygen quantity required for complete combustion of the fungus body (mol/g), a_2 is an oxygen quantity required for complete combustion of the substrate (mol/g) and a_3 is an oxygen quantity required for complete combustion of the by-products (mol/g).

If the by-product formation quantity is neglected as being small, estimation of the quantity of the fungus body in accordance with the formula (3) becomes possible because the oxygen quantity necessary for the combustion of the substrate is known and the oxygen consumption quantity of the fungus body can be obtained from the difference of the oxygen quantities at the inlet and outlet.

Estimation of the quantity of the fungus body by carbon incomings and outgoings is expressed by the following formula:

$$\triangle X = (b_2 \cdot \triangle s - 12 \cdot \triangle CO_2 - b_3 \cdot \triangle P)/b_1 \quad \ldots\ldots\ldots (4)$$

where $\triangle CO_2$ is a carbonic acid gas formation quantity (mol), b_1 is a carbon content of the fungus body (g/g), b_2 is a carbon content of the substrate (g/g) and b_3 is a carbon content of the by-products (g/g).

If the by-product formation quantity is neglected as bein small, estimation of the quantity of the fungus body in accordance with the formula (4) becomes possible because the carbon quantity of the substrate is known and the carbonic acid gas quantity of the exhaust gas can be measured.

Estimation by the multiplication model is expressed by the following formula:

$$\frac{d(VX)}{dt} = \mu VX \quad \ldots\ldots (5)$$

$$\mu = \mu_{max} \cdot \frac{S}{Ks + S} \quad \ldots\ldots (6)$$

$$\frac{d(VS)}{dt} = FS_o - \frac{1}{Y_G} \mu VX - mVX \quad \ldots\ldots (7)$$

$$\frac{dV}{dt} = F \quad \ldots\ldots (8)$$

where S is a substrate concentration in the solution (g/ℓ), μ is a specific multiplication speed (1/h), μ_{max} is a maximum specific multiplication speed (1/h), Y_G is a yield constant of the fungus body with respect to the substrate (g/g), m is a maintenance constant with respect to the substrate (g/g), Ks is a saturation constant (g/ℓ) and t is a time (h).

The quantity of the fungus body can be calculated by applying the initial solution quantity Vo, the initial fungus body concentration Xo and the coefficients μ_{max}, Ks, Y_G and m to this formula and then applying the measured value of the substrate feeding speed F.

As described above, the quantity of the fungus body can be estimated by the oxygen incomings and outgoings, carbon incomings and outgoings and multiplication model. Estimation of the quantity of the fungus body can be made more reliably in practice by combining them together.

To realize the optimal substrate feeding, the formation of the by-products must be detected. The inventors of the present invention have found that it can be detected satisfactorily by various methods such as a method using the aspiratory quotient (RQ) as the ratio of Q_{CO_2} to Q_{O_2},

a method using the flow ratio of the outlet gas to the inlet gas (hereinafter called the "β value") and a method which measures the volatile by-products in the exhaust gas such as ethanol by gas chromatography.

In the case of the enzymic incubation using sugar as the substrate, RQ = 1.0 represents complete aerobic multiplication of the fungus body, RQ = 1.0 represents the ethanol formation because the carbonic acid gas formation quantity is great and RQ = 1.0 represents that the substrate quantity is insufficient or the enzyme consumes resulting ethanol. Accordingly, the α value is reduced when RQ exceeds 1.0, assuming that ethanol is formed, and the α value is raised when RQ is considerably below 1.0, assuming that the substrate becomes insufficient.

The reason whey the ethanol formation can be detected from the ratio of the outlet gas quantity to the inlet gas quantity (β value) is that when ethanol is formed, the outlet gas quantity becomes greater than the inlet gas quantity because CO_2 is formed in a greater quantity. This fact is found by the inventors of the present invention. This method is extremely effective because it does not need the measurement of O_2 and CO_2 concentration that is necessary in the method using RQ.

The method of measuring the volatile components in the outlet gas is one that directly measures the by-products such as ethanol. Since the by-products discharged into the culture solution are scattered in the exhaust gas, they can be measured by gas chromatography or semiconductor sensors.

Examples of the substrate to be used in the present invention include glucose, fractose, succrose and molasses as the industrial raw material. Auxiliary materials are those which are generally used for incubation, such as ammonium sulfate, urea, aqueous ammonia, potassium phosphate,

yeast extract, magnesium sulfate, ferrous sulfate and various kinds of vitamines and minerals.

Figure 7 illustrates an example of the apparatus for practising the incubation method of the present invention. A seed bacteria is placed in an incubation tank 1 while the substrate is fed from a substrate tank 7 by a substrate feeding pump 8. In this case, data from an inlet oxygen partial pressure gauge 5, an inlet gas quantity meter 6, an outlet gas oxygen partial pressure gauge 11, a carbonic acid gas partial pressure gauge 12 and an exhaust gas meter 13 are applied to a computer 4 for control to calculate the quantity of the fungus body inside the tank from the multiplication model calculation on the basis of the oxygen incomings and outgoings, carbon incomings and outgoings and substrate feeding speed. The substrate feeding speed is determined from this result and from the α value and the substrate feeding pump 8 is operated in accordance with the speed thus calculated. On the other hand, RQ and the ratio of the outlet gas quantity to the inlet gas quantity (β value) are determined and the α value is changed in accordance with these values so as to control the substrate feeding speed. During incubation, the dissolved oxygen concentration must be kept at least 2 mg/ℓ and a signal of a dissolved oxygen sensor 9 is sent to a dissolved oxygen meter 10. The value of the meter 10 is then applied to the control computer 4 so as to control the number of revolution of a stirrer 2 and the inlet gas oxygen concentration and the inlet gas quantity by an oxygen separater 3. In this manner, the dissolved oxygen concentration is kept at a constant value.

Figure 8 illustrates another example of the incubation apparatus of the present invention. This apparatus makes the substrate feed control by

disposing a volatile component meter 18 in an exhaust gas line in order to raise the α value when the volatile components as the by-products are not detected, and to reduce the α value when they are detected. The construction other than that of the exhaust gas line is the same as the construction of the apparatus shown in Figure 7.

Nest, the present invention will be explained more definitely with reference to examples thereof, which are merely illustrative but not limitative in any way.

Example 1

Strain: Saccharomyces cerevisiae (bread yeast)

Medium: 500 g of glucose, 53.75 g of urea, 25 g of $Na_2HPO_4 \cdot 2H_2O$, 9.5 g of $MgSO_4 \cdot 7H_2O$, 5.5 g of KCℓ, 62.5 g of sodium citrate, 12.2 g of yeast extract, 25 mℓ of a vitamine solution and 25 mℓ of a mineral solution were dissolved in 1 ℓ of service water and the pH was adjusted to 5.0.

The vitamine solution was prepared by dissolving 0.04 g of biotin, 0.08 g of vitamine B_1, 2.0 g of vitamine B_6, 1.0 g of calcium pantothenate and 20 g of insoitol in 1 ℓ of distilled water and the mineral solution was prepared by dissolving 0.05 g of $CuSO_4 \cdot 5H_2O$, 0.8 g of $ZnSO_4 \cdot 7H_2O$ and 0.3 g of $FeSO_4(NH_4)_2 \cdot 6H_2O$ in 1 ℓ of distilled water.

Incubating condition:

50 ℓ jar fermetater; 30°C; pH 5.0. The dissolved oxygen concentration was adjusted within the range of 4 to 6 mg/ℓ by the number of revolution of a stirrer, the oxygen partial pressure and quantity of the feed gas. The oxygen partial pressure of the feed gas was changed by use of an air compressor in combination with an oxygen cylinder. The tank internal pressure was adjusted to 0.5 kg/cm^{2}G and the carbonic acid gas concentration

of the exhaust gas was adjusted within 20 %. The initial solution quantity was 15 ℓ, the initial fungus body concentration was 50 g/ℓ and the value was set to 0.3 g/g·h. The average of the fungus body quantities obtained from the oxygen incomings and outgoings and from the carbon incomings and outgoings was used as the fungus body quantity inside the incubation tank. In this case, $a_1 = 0.042$, $a_2 = 0.033$, $b_1 = 0.47$ and $b_2 = 0.40$ were used as the coefficients. The formation of ethanol as the by-products was detected by the RQ value. The α value was raised at a rate of 10 % when RQ < 0.8 mol/mol and was reduced at a rate of 50 % when RQ > 1.0 mol/mol.

Result:

The ethanol concentration could be kept below 3.4 g/ℓ throughout the 12 hours' incubation period as shown in Figure 8. The calculated value of the fungus body quantity was substantially in agreement with the measured value as shown in Figure 10. Thus, the fungus body concentration reached a high concentration of 104 g/ℓ and the yield of the fungus body was 0.43 g/g. The final culture solution quantity was 25.5 ℓ.

Example 2

Strain: Saccharomyces cervisiae (bread yeast)

Medium: Same as the medium of Example 1

Incubating condition:

The procedures of Example 1 were followed in the same way except that estimation of the fungus body quantity inside the incubation tank was carried out by the multiplication model. $\mu_{max} = 0.37$ ℓ/h, Ks = 0.0648 g/ℓ, $Y_G = 0.52$ g/g and m = 0.025 g/g were used as the coefficients of the multiplication model.

Result:

The ethanol concentration could be kept within the range of 0.15 to 4.2 g/ℓ throughout the incubation period of 10 hours. The fungus body concentration reached a high level of 100 g/ℓ and its yield was 0.43 g/g. The final culture solution quantity was 24.3 ℓ.

Example 3

Strain: Saccharomyces cervisiae (bread yeast).

Medium: Same as the medium used in Example 1

Incubation condition:

The ethanol formation was detected from the ratio of the outlet gas quantity to the inlet gas quantity (β value) and the α value was raised at a rate of 10 % when $\beta < 0.95$ and was reduced at a rate of 50 % when $\beta < 1.0$. The other conditions were the same as those used in Example 1.

Result:

The ethanol concentration could be kept below 1 g/ℓ throughout the incubation period of 10 hours. The fungus body concentration reached a high level of 105 g/ℓ and its yield was 0.44 g/g. The final culture solution quantity was 24.1 ℓ.

Example 4

Strain: Saccharomyces certivisae (bread yeast)

Medium: Same as the medium used in Example 1

Incubating condition:

The outlet gas was introduced into gas chromatography to detect the ethanol formation. The α value was raised at a rate of 10 % when ethanol was not formed and was reduced at a rate of 50 % when the ethanol concentration in the gas exceeded 1 ppm. The other conditions were the same as those

of Example 2.

Result:

The ethanol concentration could be kept below 1.4 g/ℓ throughout the incubation period of 12 hours. The ethanol concentration reached a high level of 106 g/ℓ and its yield was 0.45 g/g. The final culture solution quantity was 24.4 ℓ.

Since the present invention makes it possible to control substrate feeding while reducing the formation of by-products, the present invention provides the effect that high fungus body concentration culture of more than 100 g/ℓ can be accomplished and producibility of the incubation tank can be improved.

Claims

1. A method of controlling the feed of a substrate in aerobically incubating an enzyme, wherein the formation of by-products during incubation is detected by use of a substrate feeding speed of a substrate feed quantity to an incubation tank per unit quantity of a fungus body (specific feeding speed; hereinafter referred to as an " α value") as a parameter, siad α value is increased at a predetermined rate when no by-product is detected, and when the by-products are detected, said α value is decreased at a rate greater than said predetermined rate.

2. The method of controlling the feed of a substrate as defined in claim 1 wherein said α value is increased at a rate of up to 20 % and preferably up to 10 % when no by-product is formed and when the by-products are formed, said α value is decreased at a rate of at least 30 % and preferably 50 %.

3. The method of controlling the feed of a substrate as defined in claim 1 wherein the quantity of the fungus body inside said incubation tank is calculated from oxygen incombings and outgoings.

4. The method of controlling the feed of a substrate as defined in claim 1 wherein the quantity of the fungus body inside said incubation tank is calculated from carbon incomings and outgoings.

5. The method of controlling the feed of a substrate as defined in claim 1 wherein the quantity of the fungus body inside said incubation tank is calculated from a multiplication model based upon the substrate feeding quantity.

6. An apparatus for controlling the feed of a substrate, comprising:

a gas flow meter (6) for measuring the velocity of an inlet gas;

oxygen and carbonic acid gas analyzers for measuring

(5, 11, 12, 13) oxygen and carbonic acid gas concentration and partial pressure in inlet and outlet gases, respectively;

a substrate feeder (7) whose substrate feeding speed can be controlled on the basis of an input signal;

and

a controller (4) for performing calculation by use of the input signals from said gas flow meter (6) and from said oxygen and carbonic acid gas analyzers (5, 11, 12, 13) and applying the result of calculation to said substrate feeder (7) as its output signal.

FIG. 1A

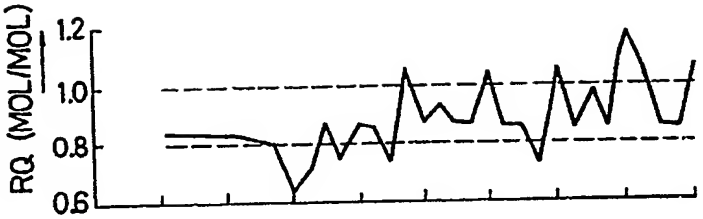


FIG. 1B

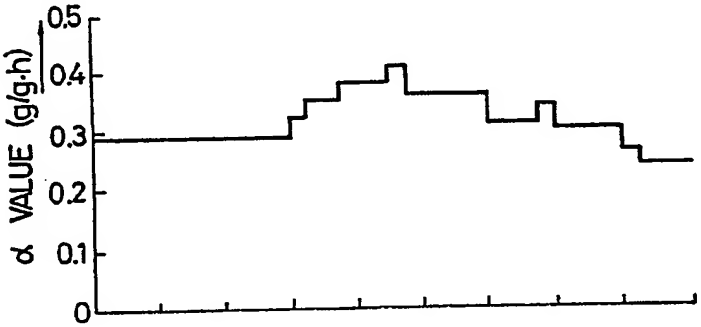


FIG. 1C

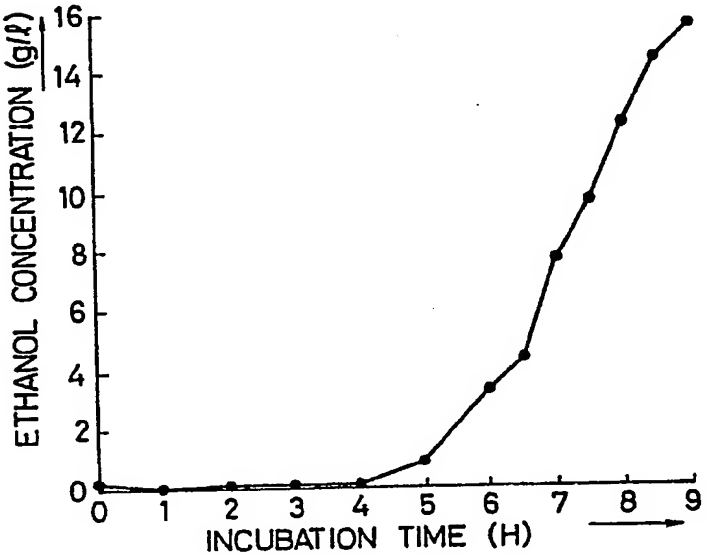


FIG. 2A

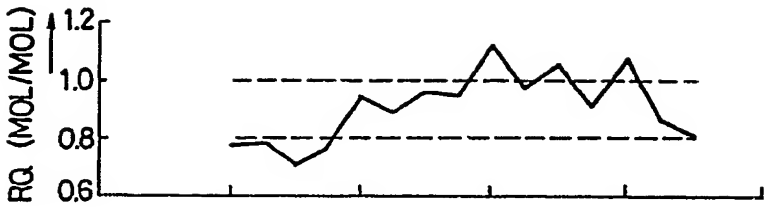


FIG. 2B

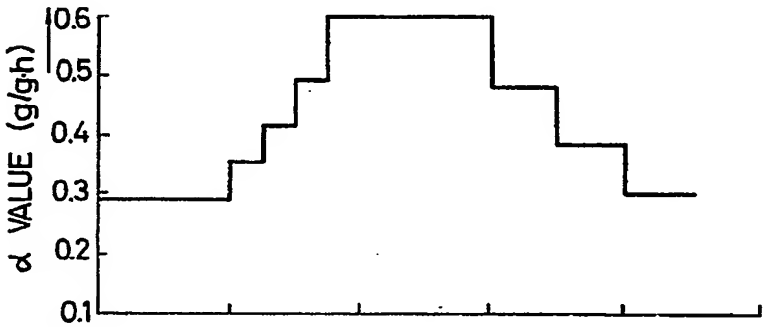


FIG. 2C

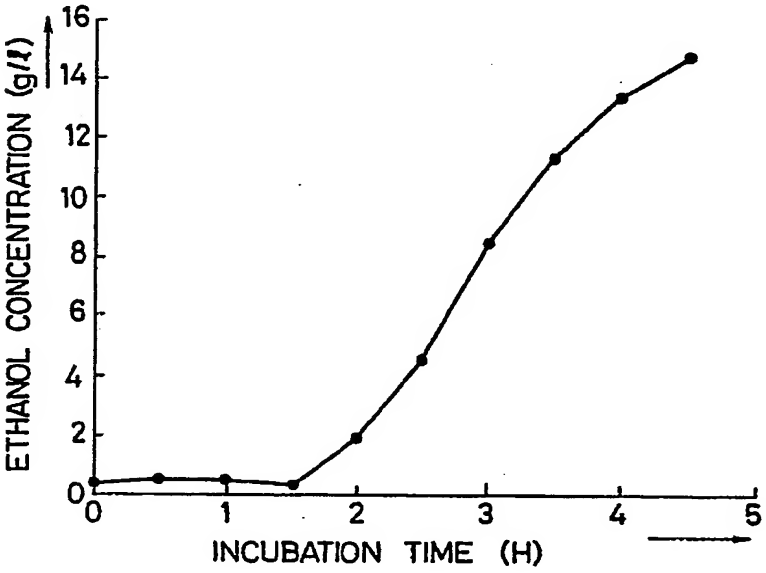


FIG. 3A

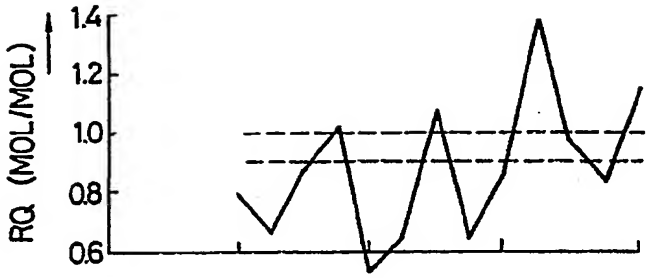


FIG. 3B

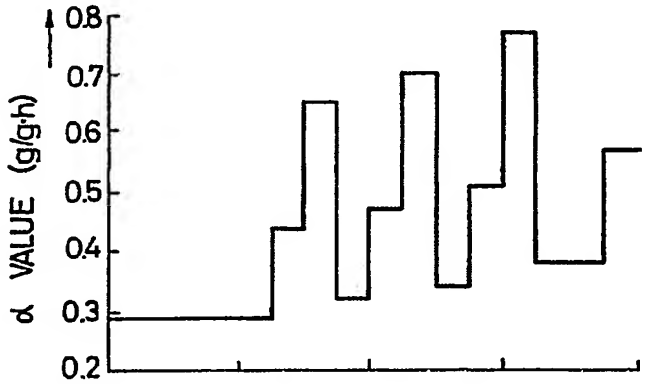


FIG. 3C

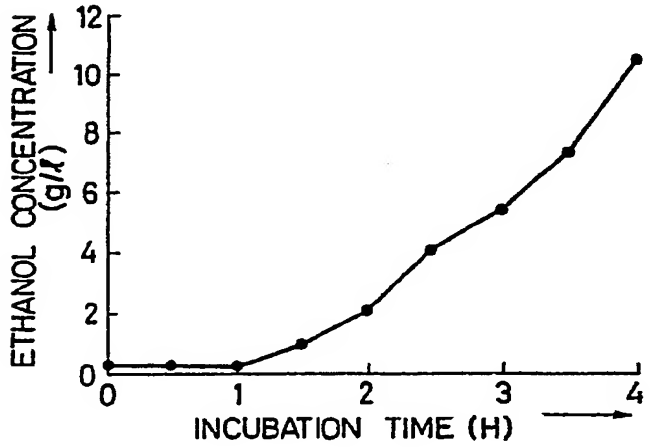


FIG. 4A

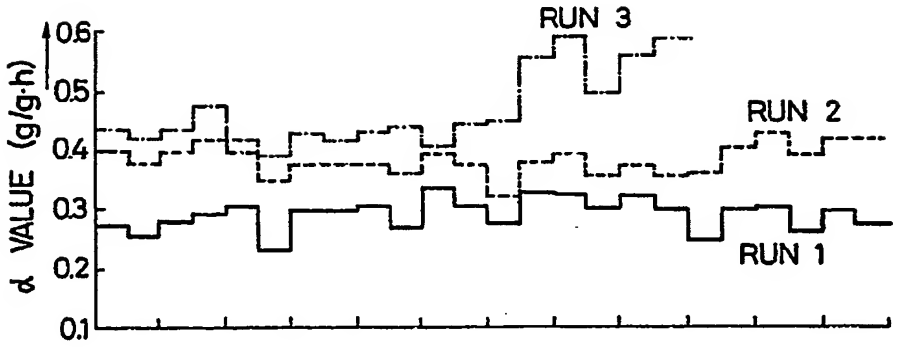

RUN 3
RUN 2
RUN 1
d VALUE (g/g·h)
0.6
0.5
0.4
0.3
0.2
0.1

FIG. 4B

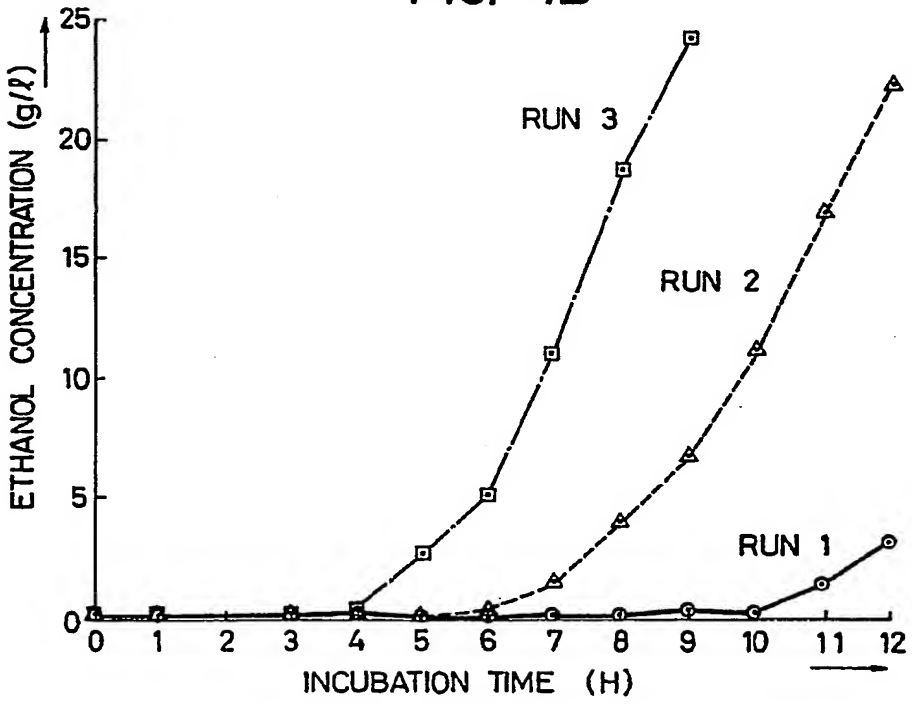

ETHANOL CONCENTRATION (g/ℓ)
25
20
15
10
5
0
RUN 3
RUN 2
RUN 1
0 1 2 3 4 5 6 7 8 9 10 11 12
INCUBATION TIME (H)

FIG. 5A

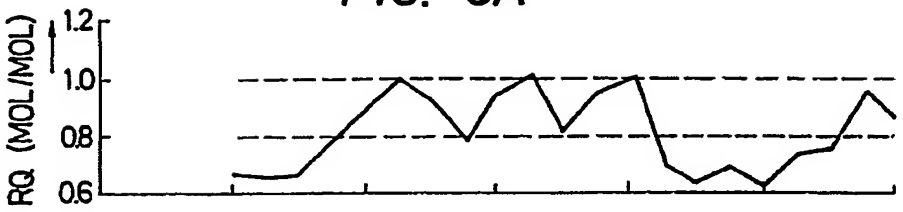


FIG. 5B

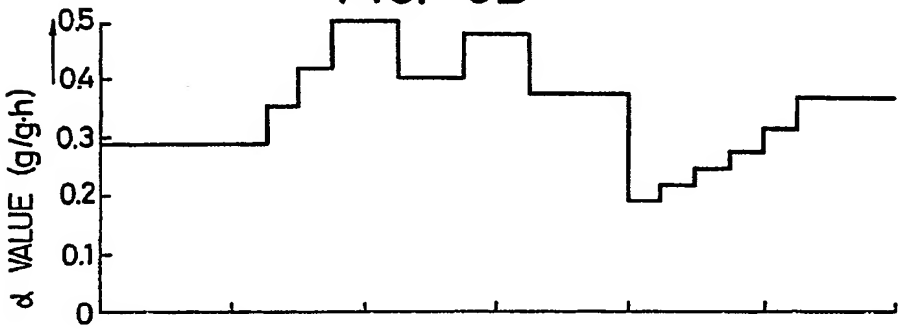


FIG. 5C

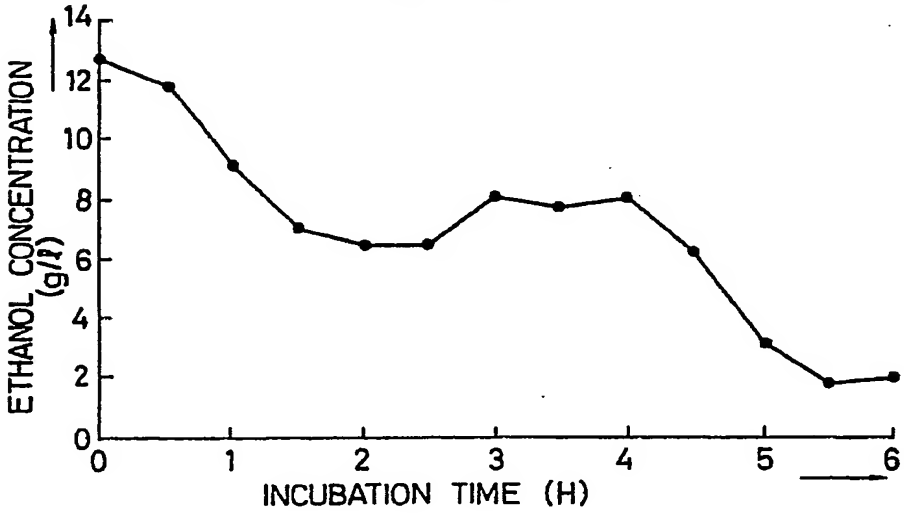


FIG. 6A

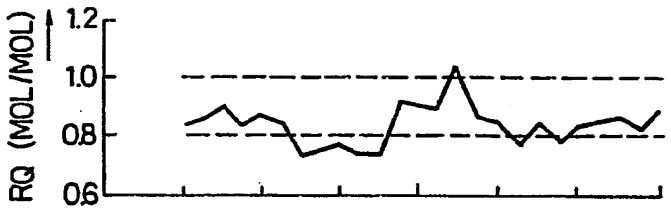


FIG. 6B

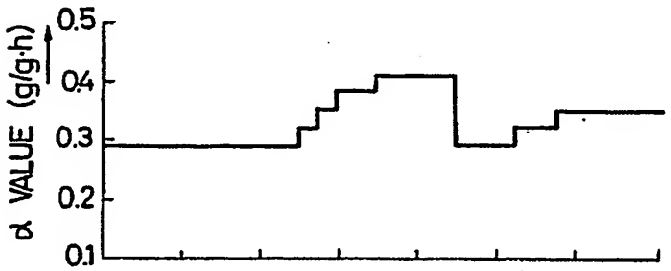


FIG. 6C

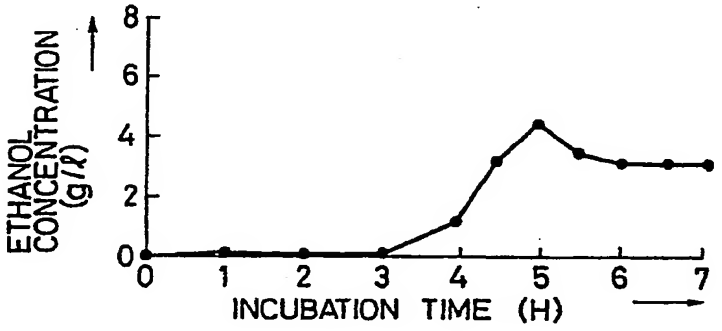


FIG. 7

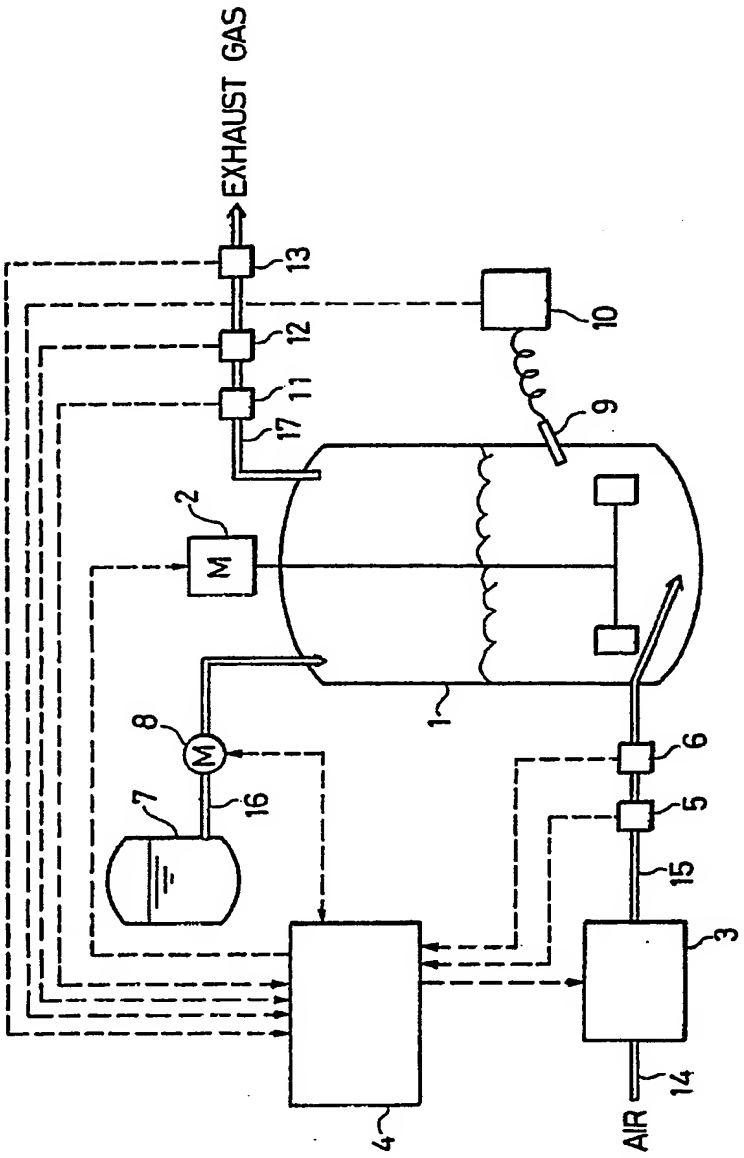


FIG. 8

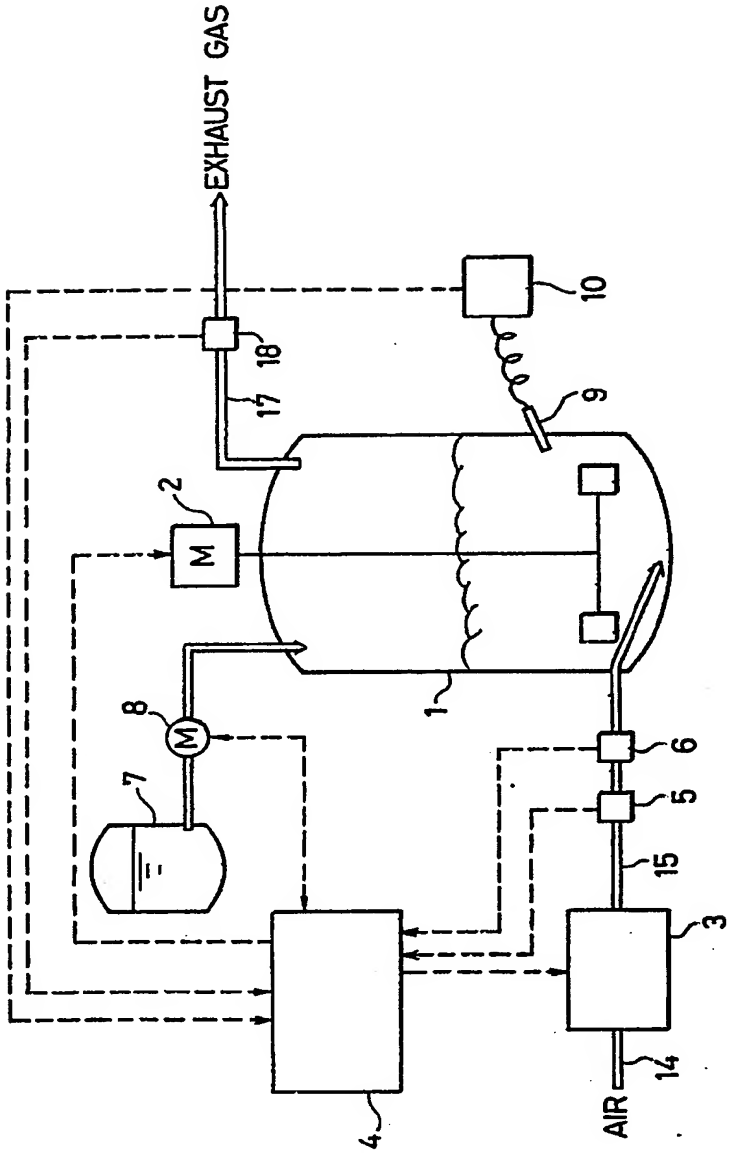


FIG. 9A

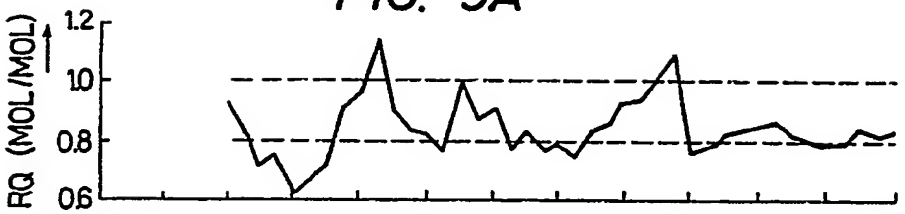


FIG. 9B

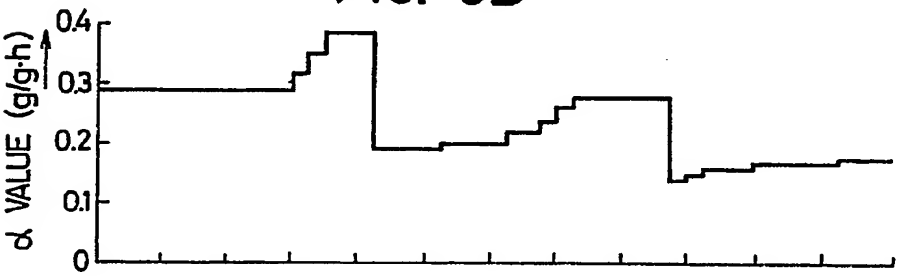


FIG. 9C

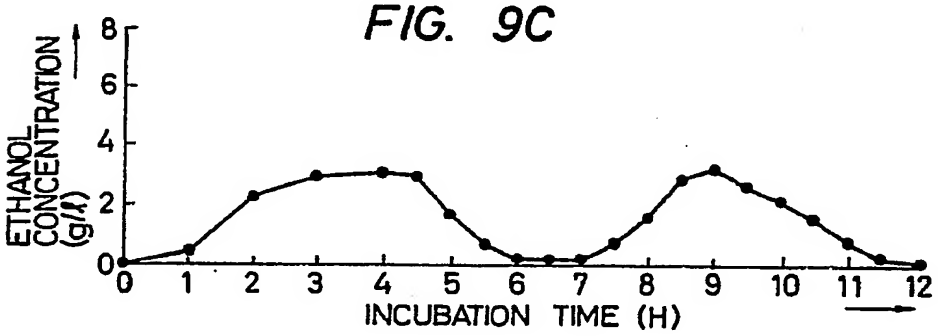
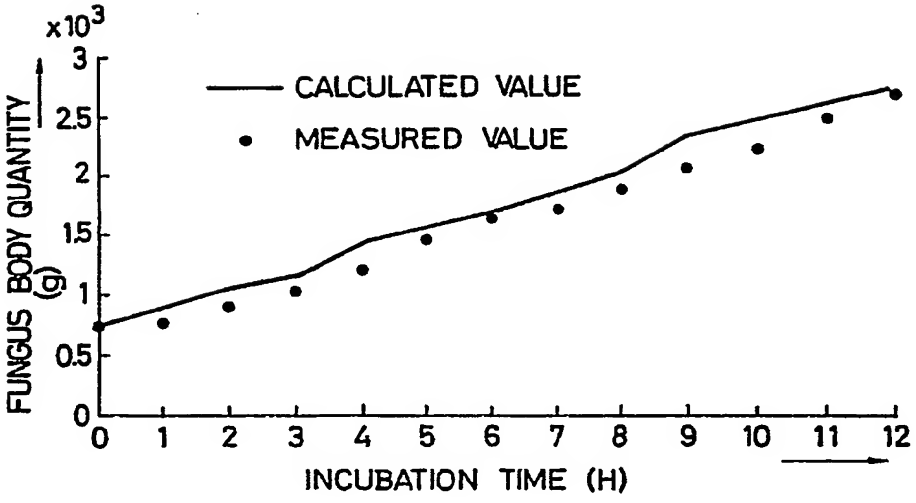


FIG. 10


x10³
3
2.5
2
1.5
1
0.5
0
FUNGUS BODY QUANTITY (g)
CALCULATED VALUE
MEASURED VALUE
0 1 2 3 4 5 6 7 8 9 10 11 12
INCUBATION TIME (H)

European Patent Office

EUROPEAN SEARCH REPORT

Application number

EP 82 10 9930

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl. ³)
X	CS-A- 181 337 (MIROSLAV RUT et al.) *Column 3, lines 3-41; column 5, lines 1-9; column 6, lines 1-9*	1,2,3	C 12 M 1/36 C 12 N 1/00 C 12 N 1/18
Y		4,5	
Y	CHEMICAL ABSTRACTS, vol. 92, no. 13, March 1980, page 512, no. 109144a, Columbus Ohio (USA); & JP - A - 79 143 584 (YAMANOUCHI PHARMACEUTICAL CO., LTD.) (08-11-1979) *Abstract*	3	
Y	FR-A-2 293 488 (P.SICK) *Page 1, lines 1-36; page 2, lines 1-25; page 3, lines 8-17; page 6, lines 26-36; page 7, lines 1-10,16-36; page 8, lines 1-34; page 9, lines 1-20; page 10, lines 1-8,17-30; page 12, lines 18-30; page 13, lines 5-36; page 14, lines 1-12; claims 1-7*	4	TECHNICAL FIELDS SEARCHED (Int. Cl. ³) C 12 B C 12 M C 12 N C 12 P

-/-

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	04-02-1983	ENGELBRECHT E

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European Patent Office

EUROPEAN SEARCH REPORT

Application number

EP 82 10 9930

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl. ³)
Y	US-A-4 167 450 (W.R.CHESBRO et al.) *Column 1, lines 10-22; column 2, lines 37-49; column 3, lines 34-68; column 4, lines 1-15,59-68; column 5, lines 1-8,38-68; column 6, lines 1-4,66-68; column 7, lines 1-4; column 8, lines 45-68; column 9, lines 1-15; claims 1,2,7,10,12,14*	5	
A		1,6	
X	FR-A-2 483 458 (A.R.ALMANZA et al.) *Page 1, lines 1-8,34-39; page 3, lines 1-4, page 3, lines 4-37; page 4, lines 1-31; page 5, lines 1-8; figure 1*	6	TECHNICAL FIELDS SEARCHED (Int. Cl. ³)
A	DE-C- 739 021 (M.SEIDEL) *Page 2, lines 1-51; page 3, lines 27-35; page 4, lines 39-122; page 5, lines 1-114*	1,6	
A	DE-B-1 174 733 (H.VON FRIES) *Entire document*	1	
A	US-A-3 002 894 (K.RUNGALDIER et al.)		

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	04-02-1983	ENGELBRECHT E

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